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IDENTITY ANALYTICS: FOR CELL-BASED THERAPIES, THE ANALYTICS, IN ADDITION TO THE PROCESS, ARE THE PRODUCT

As pharmaceutical drugs become physically larger, from small molecules to biologics to gene and cell therapies, so their molecular complexity increases. Small molecule drugs can be defined at the atomic level: it is known where each atom should be and how it should be connected to others. Methods are therefore developed to measure and define this structure, as well as those of contaminants present.

For biologics, that is, protein-based drugs, the amino acid sequence is always consistent and can be confirmed at the DNA level in the producing cell and at the protein level for the final drug. During the cell-based manufacturing process, however, chemical changes to the amino acids in the form of post-translational modifications naturally occur, leading to a somewhat discrete ensemble of closely related chemical modifications of a structure. This ensemble cannot be defined at the molecular level and to control it from batch to batch, the manufacturing process has to be controlled tightly. For biologics, therefore, 'the process is the product'.

Cell and gene therapies can be even more complex. In cases such as CAR-T cells or gene therapies, specific genes are inserted that can be quantified to judge the performance of a manufacturing process and the quality, including identity and potency, of the final product.

For the cell therapies discussed in this post, however, no gene is inserted and the adage that the process is the product is even more true. This situation requires an in-depth understanding of the analytical tools.

Given the complexity of cell therapies, changes to the method can influence the analytical outcome, without reflecting any true changes in the cell therapy being tested. Indeed, defining what cell markers uniquely identify a certain cell type is already complex. Detailed analytical development early on in the overall development process is therefore critical to success.

Non-genetically modified cell therapies

In recent years, cell therapies consisting of non-genetically modified cells have gone from development labs into the clinic. Examples include pericytes[1], mesenchymal stem cells (MSCs)[2], endothelial progenitor cells (EPCs)[3] and macrophages[4]. Each of these cell types has the potential ability to aid in the curing of a multitude of diseases. From a production point-of-view, part of the challenge has been and continues to be the definition of each of these cell types, and the establishment of robust manufacturing processes. This blog post will therefore focus on the identity markers that define specific cell types. Another important challenge is donor variability, but this is not discussed here as it has been addressed in a different, recent blog post[5].

Definition(s) of cell types

Going through the cell types mentioned highlights the challenges in first defining what constitutes the target cell. Following Cathery et al.[1], pericytes used to be defined by their anatomical location, but now cell surface markers are more often used. There is no consensus, however, on what constitutes the unique cell surface markers, although it is agreed a combination of the absence and presence of markers is needed. Commonly, markers such as neural/glial antigen 2 (NG2), proteoglycan and platelet-derived growth factor receptor-ß (PDGFRß), and CD146, together with markers such as CD90 and CD105 are present, while markers such as CD56, CD45 and CD31 are absent. More recently, pericytes or pericyte-like cells have been described as positive for CD34, NG2, PDGFRß, CD105 and CD90, while being negative for CD31, CD146 and CD45.

For mesenchymal stem cells (MSCs), a similar issue exists where defining the cells is a complex issue. Maldonado et al.[2] cite the four minimum criteria for defining MSCs from the International Society of Cellular Therapy (ISCT): (1) fibroblast-like morphology, (2) adherence to plastics; (3) capacity for differentiation into osteoblasts, adipocytes, and chondrocytes; (4) and expression of cell surface proteins CD73, CD90 and CD105, and lacking CD45, CD34, CD14, CD19, CD11b, and HLA-DR.

Endothelial progenitor cells (EPCs) appear to be even harder to define. Carolina et al.[6] define early EPCs as positive for CD45 and CD14, while also displaying some endothelial markers, while late EPCs are positive for CD13, CD34, VEGFR2 and VE-cadherin, and negative for myeloid markers. Keighron et al.[7] define early EPCs as "having an expression profile more similar to monocytes than endothelial cells" while also expressing haematopoietic markers (RUNX1, WAS, LYN) and inflammatory markers (TLRs, CD14, HLAs). Late EPCs have an expression profile similar to mature endothelial cells (CD34, VE-Cadherin, von Willebrand factor).

As a final example, macrophages are differentiated from monocytes and, depending on the details of the differentiation process, can differentiate into multiple subtypes of macrophages. One classification will differentiate between M0 (naïve), M1 (pro-inflammatory) or M2 (anti-inflammatory) macrophages. Gurvich et al.[8] investigated some of these subtypes, manufactured using protocols from different academic groups, for cell surface markers, noticing subtle differences.

They also performed a mRNA sequence analysis and showed that the different subtyptes could be distinguished from each other in a principle component analysis. Zitta et al.[9] recently added another subtype that is being tested in a clinical trial and showed that by changing only subtle aspects of the manufacturing process, such as the vessel in which the monocytes were being differentiated into macrophages, a subtype could be manufactured that was distinct from the others based on mRNA sequencing data analysis. It is not known whether these apparent different subtypes have a different clinical effect, and whether they would target different disease indications and thus require different regulatory pathways.

Implications for process development

It can be seen that the definition of the specific cell type that constitutes the 'active ingredient' in the drug can be complex. A good understanding of the biology behind the cell type, the meaning of the cell surface markers or other identity-defining characteristic, is therefore necessary and this can only be gained by having a good understanding of the biology of the cell and, ideally, the biology of the indication for which the drug is being developed. Too often, the definition is phenomenological and the value of the unique identities debatable. There is a further subtlety, especially when the identity is (largely) determined by surface markers measured by flow cytometry. The result for each marker will typically be a percentage of cells that display that marker, and for each marker a specification needs to be defined of what percentage is acceptable. Furthermore, the number or percentage of meets the specifications for all the chosen markers will be significantly smaller than for each given marker. The drug product, therefore, is never a single cell type and always a population or complex mixture of cells. The implications are considerable: what does this mean for the efficacy and safety of the product? What levels of each individual marker is acceptable? Furthermore, in our experience, regulatory agencies are therefore of the opinion that in these cases, it cannot be said that the drug consists solely of a single cell type, but rather that the drug product consists of the target cells as well as other cells derived from the same starting material.

The composition of this population of cells is controlled by the manufacturing process: by controlling the process, repeated batches should show the same characteristic distribution of cell surface markers. This consistency, rather than the absolute identity of a single cell type, contributes to the efficacy and safety of the product. The manufacturing process therefore needs to be carefully developed to be robust and repeatable.

Conversely, changes to the manufacturing process during development could result in a different distribution of cell types within the product and a comparability exercise will need to be performed to assess whether this is acceptable from the point of view of safety and efficacy.

Implications for analytical development

From the above, it can be seen that the first challenge is to define the product. An early investment into a deep understanding of the mode of action of the cells, the underlying mechanisms and pathways, is a critical part of this. This can lead to a deeper understanding of the cells and what makes them uniquely suited to perform their function. Furthermore, this can significantly inform the development of adequate methods to characterize the final product and any production intermediates. It is important that the development of the method is properly and carefully done, even at an early stage of development; and that the underlying principles of each method be understood in light of the product being tested. This is especially important in the early stages of development, as the manufacturing process is still being developed and the precise composition of the final drug product may still shift. Specifications for the markers used to define the cell identity are often based on results obtained during manufacturing, rather than on a scientific understanding of what that marker truly represents. As the manufacturing process is further developed, the levels measured in later batches may decrease and not meet the specification. Would this be considered a different product, or was the method developed and the specifications set too soon?

At 3D-PharmXchange, one of our clients encountered a similar issue, where analytical results suggesting changes had occurred across several manufactured batches. As part of a root-cause analysis, certain weaknesses were identified in how the method was set up. When these were corrected, the apparent differences disappeared and all manufactured batches were found to be comparable.

It is, therefore, important that method and manufacturing process development are done largely in parallel, and that sufficient scientific understanding, powered by orthogonal methods, is used to truly understand what is being manufactured and measured.

Conclusion

When a product is highly sensitive to the manufacturing process ('the process is the product'), and the analytics can be vague due to complex methods and sensitive dependencies on the product ('the analytics are the product'), product development to enable clinical product manufacture should be done carefully. Ideally, this should be supervised by people familiar with the product, so that suitably controlled processes and analytics can be developed to allow for entering the clinic. 3D-PharmXchange can provide valuable support in this area: we have broad experience with a variety of ATMP products, including non-genetically modified cells as discussed in here. Additionally, we have extensive method development and troubleshooting experience. When developing projects for clients, we use our experience proactively, to suitably advice the client to minimise or avoid problems in the near and more distant future of their project.

References

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